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INVESTIGATIONS IN FISH CONTROL

45. Residues of MS-222
in Northern Pike, Muskellunge, and Walleye

46. Methods of Estimating the Half-Life
of Biological Activity of Toxic Chemicals
in Water

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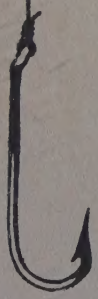

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INVESTIGATIONS IN FISH CONTROL

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By John L. Allen, Charles W. Luhning, and Paul D. Harman

46. Methods of Estimating the Half-Life of Biological Activity of Toxic Chemicals in Water

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RESIDUES OF MS-222 IN NORTHERN PIKE, MUSKELLUNGE, AND WALLEYE

By John L. Allen, Chemist
Charles W. Luhning, Technician
and Paul D. Harman, Technician
Southeastern Fish Control Laboratory
Bureau of Sport Fisheries and Wildlife
Warm Springs, Georgia 31830

ABSTRACT.--Residues of MS-222 (tricaine methanesulfonate) in muscle tissue of northern pike, muskellunge, and walleye following anesthesia were measured by a modified Bratton-Marshall colorimetric method and confirmed by thin-layer chromatography. The residues dissipate rapidly from the muscle when fish are withdrawn from the anesthetic and are near the background readings of the controls within 24 hours.

The U. S. Food and Drug Administration requires that certain disinfectants, antimicrobials, and anesthetics be cleared for their continued use on fish subject to human consumption. A part of the information necessary for clearance of MS-222 (tricaine methanesulfonate) as an anesthetic is its residues in treated fish. MS-222 has gained wide acceptance as an anesthetic for fish. The drug has been used to facilitate handling of fish during marking, spawn taking, and transporting operations.

The demand for popular species of fish such as northern pike (Esox lucius), muskellunge (Esox masquinongy), and walleye (Stizostedion vitreum vitreum) has become so great that they are extensively propagated in hatcheries. These fish are difficult to handle during such hatchery operations as spawning and marking unless they have been anesthetized. The release or use of fish treated with an anesthetic without prior knowledge of the persistance of the compound could result in consumption of an adulterated product.

Walker and Schoettger (1967a) measured MS-222 residues in four salmonids following anesthesia, and Schoettger et al. (1967) measured MS-222 residues in muscle of channel catfish (Ictalurus punctatus).

This study was undertaken to measure residues of MS-222 in the muscle of northern pike, muskellunge, and walleye.

METHODS AND MATERIALS

Fish

The investigation of MS-222 residues in northern pike, muskellunge, and walleye was performed on fish treated with MS-222 in the springs of 1967 and 1968 (table 1). The fish were treated as outlined by Schoettger and Julin (1967) and Schoettger and Steucke (1970). Samples of muscle tissue collected by personnel of the Fish Control Laboratory in La Crosse, Wis., were individually wrapped, frozen, and shipped on dry ice to the Southeastern Fish Control Laboratory, Warm Springs, Ga., for analysis. Tissue samples of all species collected in 1967 were taken by removing a portion of muscle adjacent to the dorsal fin as described by Walker and Schoettger (1967b). Northern pike muscle tissue samples collected in 1968 were

Table 1.--Species and sources of fish

Common name	Scientific name	Average weight (grams)	Year	Site of treatment 1/
Northern pike	Esox lucius	1,180	1967	NFH, Valley City, N. D.
		690	1967	NFH, Genoa, Wis.
		690	1967	FCL, La Crosse, Wis.
		1,178	1968	NFH, Valley City, N. D.
Muskellunge	Esox masquinongy	544	1967	NFH, New London, Minn.
		770	1967	NFH, Valley City, N. D.
Walleye	Stizostedion vitreum vitreum	1,286	1967	NFH, Genoa, Wis.
		1,286	1967	FCL, La Crosse, Wis.

1/ NFH = National Fish Hatchery; FCL = Fish Control Laboratory.

taken by filleting the fish and homogenizing the entire fillet before analysis.

Analysis

The samples from northern pike, muskellunge, and walleye treated in 1967 were analyzed by the modified Bratton-Marshall colorimetric method of Walker and Schoettger (1967b). In addition, at least one fish from each withdrawal interval was analyzed according to the thin-layer chromatographic method of Allen, Luhning, and Harman (1970), except that a developing solution of 2-percent methanol in benzene was used.

The samples of northern pike collected in 1968 were taken from homogenized fillets and were analyzed by the colorimetric method of Walker and Schoettger (1967a). Each sample was analyzed also by the ancillary thin-layer chromatographic (TLC) method of Allen, Luhning, and Harman (1970).

Although the colorimetric method provides the accuracy of a photometric measurement, background readings from naturally occurring primary aromatic amines prevent the detection of MS-222 residues less than background. The thin-layer chromatographic procedure was used to identify the residues of MS-222 and to indicate when the residues had decreased to a concentration below the sensitivity of the procedure, which is approximately 0.2 ppm. Residues of less than 2.0 ppm are reported as a trace. The R_f of the sample spot was compared with the R_f of an MS-222 standard on the same plate to identify the residues as MS-222.

RESULTS

Northern pike

Residues of free MS-222 in northern pike treated in 1967 were measured by colorimetric analysis and averaged 8.4 ppm in the 0-hour withdrawal at 12° C to within the background readings of the controls at 24-hour withdrawal. The fish in the colder water accumulated higher residue concentrations at 0-hour than those in the warmer water. At all temperatures the 24-hour withdrawal samples were within the background readings of the controls. No residues of MS-222 could be detected even by the more selective thin-layer chromatographic analysis after 24 hours withdrawal (table 2). Residues of MS-222 were confirmed by thin-layer chromatography only in samples showing colorimetric readings higher than the control samples. One control sample showed a trace of MS-222 by thin-layer chromatography, but this sample may have been contaminated with MS-222 during

Table 2:--Residues of MS-222 including background aromatic amines in muscle tissue of northern pike at selected temperatures and withdrawal intervals following deep anesthesia with a 150 ppm solution of MS-222

Temperature and withdrawal interval	Site and year of treatment	Number of fish analyzed 1/	Bratton-Marshall method--residues in ppm				TLC method -- estimated free MS-222 residues in ppm 2/
			Free MS-222		Free and acetylated MS-222		
			Mean	Range	Mean	Range	
8° C							
Control	Valley City NFH 1967	3 (1)	0.7	0.1- 1.8	2.0	1.5- 2.4	ND
0-hour	Do 1967	3 (1)	10.9	5.4-16.0	11.3	5.4-18.0	7.0
2-hour	Do 1967	3 (1)	5.3	2.4- 9.0	6.2	4.8- 8.4	4.0
24-hour	Do 1967	3 (1)	0.8	0.0- 1.2	1.3	0.8- 2.0	ND
12° C							
Control	Genoa NFH 1967	3 (1)	1.2	0.4- 2.8	2.6	2.0- 3.0	Trace
0-hour	Do 1967	3 (1)	8.4	3.2-17.0	9.1	5.4-15.0	6.7
2-hour	Do 1967	3 (1)	2.9	1.6- 4.6	5.7	4.0- 7.0	4.0
24-hour	Do 1967	3 (1)	0.8	0.5- 1.0	2.0	1.5- 2.4	ND
17° C							
Control	La Crosse FCL 1967	3 (3)	1.0	0.0- 2.4	2.1	1.5- 2.8	ND
0-hour	Do 1967	3 (1)	7.3	3.0-10.6	8.3	2.8-13.0	6.7
2-hour	Do 1967	3 (1)	0.7	0.0- 2.0	0.5	0.0 - 1.0	ND
6-hour	Do 1967	3 (2)	0.3	0.0- 1.0	0.5	0.1- 1.0	ND
24-hour	Do 1967	2 (2)	1.1	0.0- 2.2	1.0	1.0- 1.0	ND
12° C							
Control	La Crosse FCL 1968	3 (3)	0.1	0.0- 0.2	1.4	1.0- 1.6	ND
0-hour	Do 1968	3 (3)	19.5	12.0-30.0	22.0	16.8-28.0	14.0
6-hour	Do 1968	3 (3)	0.1	0.0- 0.2	0.7	0.2- 1.0	ND
24-hour	Do 1968	3 (3)	0.0	0.0- 0.0	0.7	0.6- 1.0	ND
17° C							
Control	La Crosse FCL 1968	3 (3)	0.1	0.0- 0.2	1.8	1.4- 2.4	ND
0-hour	Do 1968	3 (3)	9.4	6.0-12.0	12.2	9.0-15.0	9.4
6-hour	Do 1968	3 (3)	1.6	1.0- 2.4	2.0	1.6- 2.8	Trace
24-hour	Do 1968	3 (3)	0.7	0.2- 1.0	1.1	1.0- 1.4	Trace 3/

1/ Number in parentheses indicates the number of fish analyzed by the TLC method.

2/ ND = None detected; Trace = less than 2.0 ppm.

3/ Two of the three fish in this group showed a trace of MS-222 residue, but no residue was detected in the third fish.

analysis. There was not enough sample remaining to repeat the analysis.

In 1968, northern pike were anesthetized to medulary collapse with 150 ppm of MS-222 at 12° and 17° C at the Fish Control Laboratory at La Crosse, Wis. Residues of free MS-222 measured by colorimetric analysis averaged 19.5 ppm at 0-hour withdrawal to within the background readings of the controls at 6-hour withdrawal in fish treated at 12° C. Thin-layer chromatographic analysis of each fish from each withdrawal interval confirmed the presence of MS-222 residues only at the 0-hour withdrawal interval (table 2). At 17° C, residues of free MS-222 determined by colorimetric analysis averaged 9.4 ppm at 0-hour to trace after 24 hours withdrawal from the anesthetic. Thin-layer chromatography confirmed the presence of MS-222 residues in all the fish treated at this temperature with the exception of one fish from the 24-hour withdrawal interval.

Muskellunge

Residues of free MS-222 in muskellunge anesthetized to medullary collapse with 150 ppm of MS-222 at 10° C at the New London National Fish Hatchery in Minnesota averaged 7.9 ppm at 0-hour withdrawal, decreasing to background during 24 hours of recovery in fresh water (table 3). Thin-layer chromatographic analysis of two fish from each withdrawal period confirmed the presence of MS-222 residues at the 0-hour and 2-hour withdrawals. No residues were detected at the 24-hour withdrawals by TLC.

Walleye

In walleye treated at 17° C with 100 ppm of MS-222, residues of free MS-222 averaged 2.4 ppm at 0-hour withdrawal, and had decreased to within the background readings of the control samples during 24 hours of recovery in fresh water (table 4). Thin-layer chromatography of one fish from each withdrawal interval confirmed the presence of MS-222 residues in fish from all withdrawal intervals except those at 24 hours. The 6-hour withdrawal sample showed only a trace of MS-222 residue remaining.

Walleye exposed to 120 ppm of MS-222 at 10° C at the Genoa National Fish Hatchery in Wisconsin showed residues of free MS-222 at 0-hour that averaged 4.3 ppm and decreased to within the background levels of the controls after 24 hours in fresh water (table 4). Thin-layer chromatography of one fish from each withdrawal interval confirmed the presence of MS-222 at each withdrawal interval except at 24 hours.

DISCUSSION

MS-222 residues remaining after 24 hours withdrawal from the drug were shown to vary little from the background amines of the controls as detected by colorimetric analysis. The colorimetric analysis for total free plus acetylated aromatic amines generally showed only slightly higher concentrations than the analysis for free aromatic amines only. Certain samples showed slightly higher readings for free MS-222 than for both free and acetylated aromatic amines. It is possible that small amounts of some of these amines could have been lost during the digestion step.

The identification of the residues was made by comparing the R_f of the spot obtained from the sample to the R_f of the spot given by the MS-222 standard. The semi-quantitation of the residues by thin-layer chromatography was accomplished by comparing the size and intensity of the sample spot with a series of standard spots of known concentrations. The quantitative estimates made by thin-layer chromatography were in agreement with the results obtained by colorimetric analysis. This indicates that most of the aromatic amine above the background reading of the controls detected by the colorimetric method was MS-222.

Table 3:--Residues of MS-222 including background aromatic amines in muscle tissue of muskellunge at selected withdrawal intervals following deep anesthesia with a 150 ppm solution of MS-222 at 10° C

Withdrawal interval	Site of treatment	Bratton-Marshall method--residues in ppm: Free MS-222 Mean	Free MS-222 Range	Free and acetylated MS-222 Mean	Free and acetylated MS-222 Range		TLC method--estimated free MS-222 residues in ppm (mean) 1/ 2/	
Control	New London NFH	0.3	0.1- 1.0	1.2	1.0- 1.5	(3)	ND	(2)
0-hour	Do	7.9	3.4-10.5	10.7	7.6-13.0	(3)	4.3	(2)
2-hour	Do	0.8	0.2- 1.0	2.2	1.5- 2.8	(3)	Trace	(2)
24-hour	Do	0.4	0.0- 1.0	2.0	1.0- 2.8	(3)	ND	(2)

1/ Number of fish in parentheses.
2/ ND = None detected; Trace = less than 2.0 ppm.

Table 4:--Residues of MS-222 including background aromatic amines in muscle of walleye at selected concentrations, temperatures, and withdrawal intervals following deep anesthesia

Temperature, concentration, and withdrawal interval	Sites of treatment	Bratton-Marshall method--residues in ppm: Free MS-222 Mean	Free MS-222 Range	Free and acetylated MS-222 Mean	Free and acetylated MS-222 Range		TLC method--estimated free MS-222 residues in ppm 1/ 2/	
10° C, 120 ppm								
Control	Genoa NFH	1.0	0.6-2.0	1.1	0.3-2.0	(3)	ND	(1)
0-hour	Do	4.3	1.0-7.6	3.6	0.5-7.6	(3)	8.0	(1)
2-hour	Do	1.8	0.4-4.0	1.0	1.0-1.0	(3)	Trace	(1)
6-hour	Do	1.6	0.4-4.0	0.4	0.0-0.8	(3)	Trace	(1)
24-hour	Do	0.4	0.0-1.0	1.0	0.5-2.2	(3)	ND	(1)
17° C, 100 ppm	La Crosse FCL							
Control	Do	1.7	0.4-2.8	2.3	2.0-2.8	(3)	ND	(1)
0-hour	Do	2.4	0.4-3.6	2.5	1.0-3.6	(3)	4.0	(1)
2-hour	Do	2.8	1.0-5.0	3.3	2.0-5.4	(3)	Trace	(1)
6-hour	Do	2.0	1.4-2.8	2.5	2.0-2.8	(3)	Trace	(1)
24-hour	Do	1.3	1.0-2.0	2.3	1.2-3.4	(3)	ND	(1)

1/ Number of fish in parentheses.
2/ ND = None detected; Trace = less than 2.0 ppm.

With the exception of two samples of northern pike (table 2), no MS-222 residues were detected by thin-layer chromatography in fish withdrawn from the drug for 24 hours. Two of the three northern pike treated at 17° C and withdrawn from the anesthetic for 24 hours in 1968 showed a trace of MS-222 by thin-layer chromatography, but no residue was detected in the third sample. The lower limit of sensitivity of the thin-layer chromatographic procedure is 0.2 µg of MS-222, but quantitation generally is not possible in samples spiked at less than 2.0 ppm.

Thus a trace of MS-222 as detected by TLC indicates the presence of MS-222 at concentrations of 2.0 ppm or less. The concentration of free MS-222 including background amines for the two 24-hour withdrawal samples treated at 17° C in 1968 was 1.0 ppm by colorimetric analysis. Both the thin-layer chromatographic and colorimetric method showed that these two samples contained less than 2.0 ppm of MS-222 residue.

Walleye were treated at a lower concentration of MS-222 and had the lowest concentration of MS-222 residues at 0-hour withdrawal. Northern pike had the highest concentration of MS-222 residues at 0-hour withdrawal. At the end of 24 hours, however, there were no significant differences in residue concentrations in any of the samples.

SUMMARY

Residues of MS-222 in muscle of northern pike, muskellunge, and walleye following anesthesia at selected temperatures were measured by the modified Bratton-Marshall colorimetric method (Walker and Schoettger, 1967b) and confirmed by thin-layer chromatography (Allen, Luhning, and Harman, 1970).

The mean concentration of MS-222 residues at the 0-hour withdrawal interval ranged from 2.4 to 19.5 ppm. The level of MS-222 residues at 0-hour withdrawal was highest in northern pike and lowest in walleye. After 24 hours withdrawal from the anesthetic, residues were detected in only 2 of the 12 northern pike analyzed by thin-layer chromatography, and these two samples were shown to contain less than 2.0 ppm.

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INVESTIGATIONS IN FISH CONTROL

46. Methods of Estimating the Half-Life of Biological Activity of Toxic Chemicals in Water

By Leif L. Marking

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METHODS OF ESTIMATING THE HALF-LIFE OF BIOLOGICAL ACTIVITY OF TOXIC CHEMICALS IN WATER

By Leif L. Marking, Chemist
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

ABSTRACT.--In the absence of analytical methods, the half-life of biological activity of a chemical (the time required to decrease its toxic activity by one-half) can be estimated by bioassays. The methods presented require the determination of LC50 values (concentration producing 50% mortality) for organisms in aged solutions containing unknown residual concentrations, and concurrent tests of solutions containing known concentrations. The half-life of biological activity is determined by plotting (1) the percent concentrations remaining in aged solutions or (2) the deactivation indices against aging time on cyclic semilogarithmic graph paper. By the first method, a 5-day half-life for antimycin, a fish toxicant, was determined using resistant channel catfish (Ictalurus punctatus) and goldfish (Carassius auratus) and for sensitive green sunfish (Lepomis cyanellus) and rainbow trout (Salmo gairdneri) in soft water at pH 7.5. The second method, using only rainbow trout, also estimated a 5-day half-life for antimycin in soft water at pH 7.5. These methods could be used also to determine the half-life of antimycin at different pH's, temperatures, and light intensities, the factors thought to influence the toxicant's efficacy. The methods are not limited to fish and fish toxicants.

The biological activity of a toxicant is the killing power, which decreases as the toxicant is deactivated biologically and chemically with time. The half-life of a toxicant's biological activity is the time necessary for that activity to be reduced by one-half. Regulatory agencies now require data on the persistence of toxicants in the environment, and the half-life or rate of disappearance is a good measure of persistence.

The half-life of most fish toxicants in water is influenced by pH, temperature, sunlight, metabolism by aquatic organisms, and other environmental variables.

Analytical methods to detect and measure very small concentrations of some fish toxicants are unavailable. For example, antimycin, a fish toxicant, kills rainbow trout at 30 parts per trillion (Berger et al., 1969), but analytical methods aren't available to quantitate this concentration. Walker et al. (1964) observed that antimycin degrades rapidly in water, especially under alkaline conditions, and recommended further definition of the detoxification rate.

In lieu of inadequate analytical methods, I have outlined two laboratory bioassay methods, free of analytical instrumentation, to estimate the half-life of biological activity for toxicants used in water. The first method requires test animals that are differentially sensitive to a toxicant in order to quantitate the decreased concentrations resulting from degradation. The second method requires one test species

and greater than lethal concentrations of a chemical that are aged to levels commensurate with the tolerance of the selected species.

Mortalities in the bioassays with degrading concentrations are compared with those in concurrent reference tests on the same organism using known concentrations of the toxicant. The experiments with antimycin included sensitive and resistant species of fish, but other aquatic organisms such as mollusks, aquatic insects, and plants may be used in the bioassays for the half-life determination. The intra-species tolerance differences are of little consequence in the procedure since a reference bioassay is conducted concurrently with the degrading solutions.

The half-life of biological activity of a toxicant can be determined for various environmental parameters such as pH and temperature. Such information would be invaluable to fishery biologists in selecting minimum lethal concentrations of fish toxicants or aquatic herbicides for target species and in avoiding hazards to nontarget species. Field biologists using antimycin have reported incomplete kills of fish that probably resulted more from rapid deactivation of the antibiotic in water of a particular quality than from applying a nontoxic concentration. For example, 1 ppb of antimycin may kill all of the target fish in water of pH 8, but fail to kill any in water of pH 9. Knowledge of chemical persistence of toxicants in the aquatic environment is essential for efficacious, safe, and economical control of target organisms.

METHODS

The fish toxicant antimycin was chosen as an example to determine the half-life of biological activity according to the methods described. Previous reports indicate that the toxicity of antimycin is influenced by chemical and biological properties of the water, especially those controlling pH, but no one has been able to quantify the rate of deactivation. The static bioassay routine and maintenance of test fish were essentially those described by Lennon and Walker (1964) and Hunn et al. (1968). All the tests were conducted in soft water at pH 7.5 and 12° C.

Method A

The concentration of biologically active chemicals remaining in solutions permitted to age for a selected period of time can be estimated by introducing fish or other susceptible aquatic organisms for bioassay. More resistant species are exposed to lethal solutions of toxicants that have been aged slightly, whereas more sensitive species are exposed to older solutions that have become deactivated to lower concentrations. Concurrently with tests in aged media, identical organisms must be employed in bioassays of fresh solutions under identical test conditions and exposure periods at concentrations expected to produce mortality. The mortality data from the tests in aged and fresh solutions are analyzed to determine the statistical LC50 (concentration producing 50 percent mortality) according to the method of Litchfield and Wilcoxon (1949). The LC50's are calculated for both tests on the basis of applied concentrations even though the concentration has decreased in the aging tests.

To determine the approximate concentration remaining in the aged bioassays, a formula was developed using hypothetical data for ideal disappearance of biological activity presented in table 1. The hypothetical data are characteristic for a toxicant with a half-life of 1 day. LC50's for all of the tests in aged solutions (C_1) are identical at 50 ppb in this hypothetical case. In actuality, the statistical LC50 would not be consistent for each test, but I have kept it constant for simplicity. The actual concentrations remaining in the aged

solutions are unknown after aging begins, yet the LC50's are calculated from the concentrations originally applied to the test water. In reference tests with fresh solutions (C_2), the concentrations required decrease proportionately with the deactivation of aged solutions, and therefore more sensitive species are required to conform to these lower concentrations. The percent concentration remaining is calculated according to the following equation:

$$\text{Percent concentration remaining} = 100 - \left[\frac{(C_1 - C_2)}{C_1}\right] 100$$

Where C_1 = LC50 of aged solutions

C_2 = LC50 of fresh solutions

This computation is repeated for each aging time interval.

A plot of the percent concentration remaining and aging time (table 1) on semilogarithmic graph paper reveals a typical first-order decay curve with a half-life of 1 day. The unit of days was chosen for the hypothetical situation, but hours or weeks are appropriate when toxicants are deactivated slower or faster.

Example

Four series of uniform concentrations of antimycin were set up and permitted to age prior to introducing the test species. Each series included concentrations of 20, 40, 60, 80, and 100 ppb of antimycin and a control with no toxicant. After 7 days of aging, 10 channel catfish were introduced into each concentration and the control vessel of a single series. On the same day, antimycin was added to five vessels containing 10 channel catfish each at concentrations ranging from 1 to 40 ppb. The 96-hour LC50 of the 7-day-old solutions was calculated to be 47.300 (C_1), and that value for the fresh solutions is 20.400 ppb (C_2). By substituting the values of C_1 and C_2 into the formula, the percent concentration remaining after 7 days is calculated to be 43.13 (table 2).

Goldfish were added to the second series of antimycin concentrations of 20, 40, 60, 80, and 100 ppb after the solutions had aged for 30 days. Concurrently, goldfish were bioassayed in fresh solutions of antimycin, and the 96-hour LC50's were computed for both tests. The resulting percent concentration remaining after 30 days is 1.10 (table 2).

Green sunfish were added to another series of aged solutions after 35 days of deactivation, and rainbow trout after 44 days of deactivation. The percent concentration remaining was computed for both species (table 2).

The reference LC50's (C_1) show different sensitivity among the four species, and the 96-hour LC50's range from 20.400 ppb for the more resistant catfish to 0.049 ppb for the more sensitive trout. The LC50's are characteristic for these species in this water quality, but change in tests using different water.

The percent concentration remaining and aging time from table 2 are plotted on cyclic semilogarithmic graph paper (fig. 1). The curve approximates a first-order decay curve and describes the rate of disappearance for antimycin in soft water of pH 7.5. The percent concentration remaining is 50 after 5 days and 25 after 10 days; therefore the half-life of biological activity is 5 days.

Method B

The half-life of biological activity can be estimated by using only one species of test organism. The procedure requires aged solutions of toxicant, but stronger solutions are established and allowed to age for longer periods, such as 2, 4, 8,

Table 1:--Percent of concentrations remaining as calculated from hypothetical data on aged and fresh solutions of toxicant

Aging time for C_1 (days)	LC50 1/ (ppb) of Aged solutions (C_1)	LC50 1/ (ppb) of Fresh solutions (C_2)	Percent reduction of LC50	Percent concentration remaining
0	50.000	50.000	0.00	100.00
1	50.000	25.000	50.00	50.00
2	50.000	12.500	75.00	25.00
3	50.000	6.250	87.50	12.50
4	50.000	3.125	93.75	6.25
5	50.000	1.563	96.87	3.13
6	50.000	0.782	98.43	1.57
7	50.000	0.391	99.21	0.79
8	50.000	0.196	99.60	0.40
9	50.000	0.098	99.80	0.20
10	50.000	0.049	99.90	0.10

1/ Calculated from original concentrations of aged and fresh solutions bioassayed concurrently.

Table 2:--Toxicity of antimycin to fish in aged and fresh solutions at pH 7.5 and 12° C.

Species	Age in days of aged test solutions	96-hour LC50 (ppb) of aged (C_1) solutions	96-hour LC50 (ppb) of fresh (C_2) solutions	Percent concentration remaining
Channel catfish (*Ictalurus punctatus*)	7	47.300	20.400	43.13
Goldfish (*Carassius auratus*)	30	60.500	0.650	1.10
Green sunfish (*Lepomis cyanellus*)	35	52.000	0.308	0.59
Rainbow trout (*Salmo gairdneri*)	44	34.400	0.049	0.20

16, 32, or more days. As the solutions deactivate to a level of toxicity commensurate with the tolerance of the test organism, they are bioassayed against the test species. Concurrently with the bioassay of aged solutions, fresh solutions are bioassayed against individuals of the same species to provide reference data.

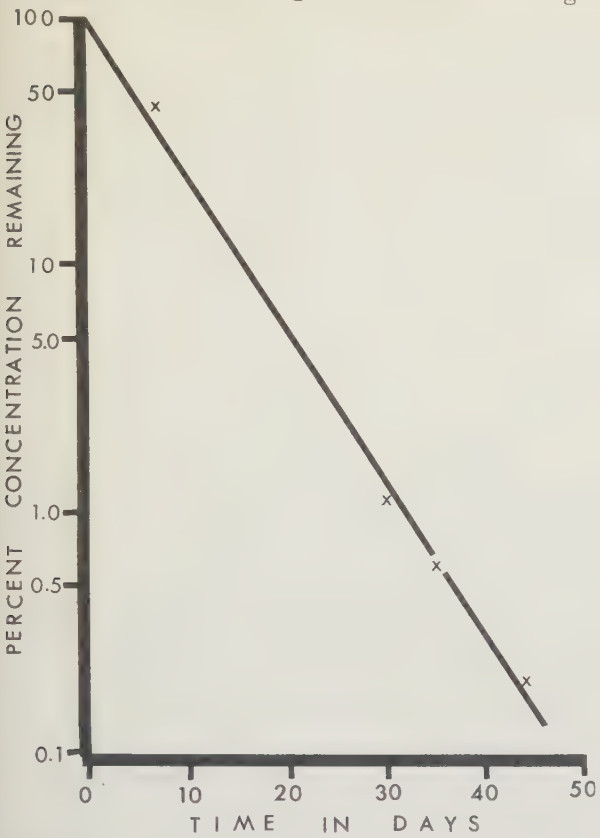

Figure 1:--The inactivation of antimycin at pH 7.5 and 12° C by method A.

The results obtained by bioassaying aged and fresh solutions against a single species are used to compute a deactivation index. The deactivation index is derived by dividing the LC50 of an aged solution by the LC50 of a fresh reference solution. The index has a value greater than 1 if the chemical deactivates at all. A value of 2 indicates that the concentration of an aged solution has diminished by one-half. The aging time required for a deactivation index of 2, calculated from the LC50's, is equivalent to the half-life of biological activity of the toxicant.

The deactivation index invariably does not equal exactly 2 for any particular test. Therefore, the deactivation index is computed for each aging period, and the results are plotted on semilogarithmic coordinates. The slope of the curve influences the half-life value and is derived from a line fitting the plotted deactivation indices.

Example

Rainbow trout were selected to assess this alternate method for half-life of biological activity. Five series of different concentrations of antimycin were prepared on different days during a 16-day period. The first series of solutions contained higher concentrations than subsequent series because it would be deactivating for 16 days prior to introducing the fish. The next series aged for 8 days, and the initial concentrations were lower than in the first series. Another series of concentrations was aged 4 days, and another for 2 days prior to introducing the trout. All of the aged solutions were bioassayed on the same day. Concurrently, a reference bioassay with rainbow trout was conducted using known concentrations in fresh solutions. The 96-hour LC50's for solutions aged for different time periods were used to compute the deactivation indices (table 3).

The deactivation indices range from 1.25 to 7.5 and signify the deactivation occurring over the test period. They are plotted against aging time on semilogarithmic graph paper (fig. 2). From this graph, the half-life of biological activity for antimycin in soft water at pH 7.5 and 12° C is about 5 days. This value agrees with that determined by method A.

DISCUSSION AND CONCLUSIONS

The model (table 1) conveniently presents data for each 1-day interval of the aging process. Quite frequently, as in the example of antimycin, species are unavailable for consistent 1-day increments and consequently the aging times before introducing the species do not coincide with the half-lifes. This does not prevent the half-life determination, however, since the

Table 3:--Toxicity of antimycin (ppb) to rainbow trout in aged (C_1) and fresh (C_2) solutions in 96-hour tests at pH 7.5 and 12° C.

Aged solutions		Fresh solutions	Deactivation index
Age in days	LC50 (C_1)	LC50 (C_2)	
2	0.0498	0.040	1.25
4	0.0787	0.040	1.97
8	0.130	0.040	3.25
16	0.300	0.040	7.50

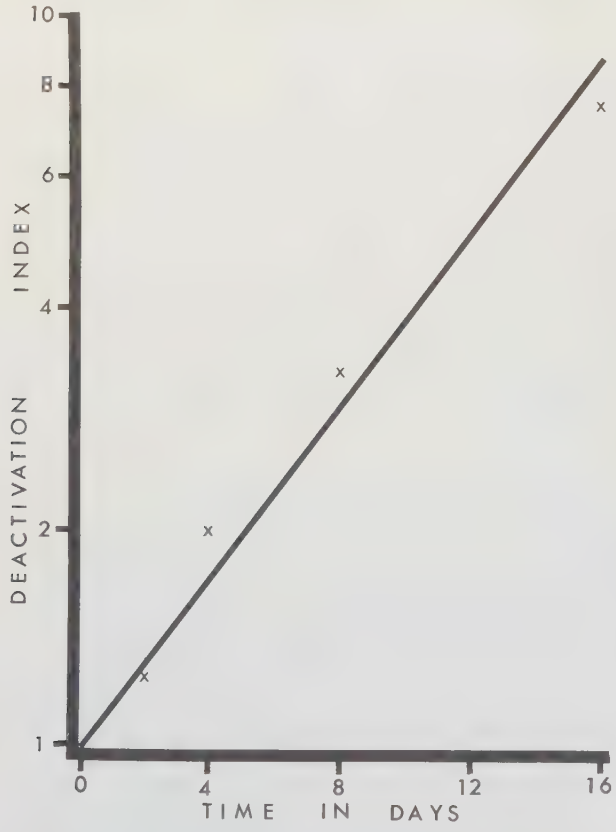

Figure 2:--The inactivation of antimycin at pH 7.5 and 12° C by method B..

percent reduction can be plotted at any time a species can be found which is sensitive to the aged solutions.

In field applications, target organisms must be exposed to a minimum lethal concentration or greater for a certain period of time for successful eradications. Knowledge of the half-life of biological activity permits selection of concentrations which will retain sufficient toxicity throughout an exposure period to kill the target organism. In addition, the concentration remaining in the treated water can be calculated for any reasonable length of time. For instance, highly sensitive species may be introduced into waters in which highly resistant species were eradicated. The time required for the toxicant to become sublethal to the sensitive species can be extrapolated from the half-life curve.

In the event that a chemical detoxifies too slowly and bioassay space and equipment cannot be tied up for long periods, the procedure for method A may be divided into segments which can be accomplished concurrently. For example, resistant species may be tested in aged (C_1) and fresh (C_2) solutions while at the same time sensitive species may be tested in lower concentrations of aged (C_1) and fresh (C_2) solutions. The percent reduction in concentrations for each time segment is plotted on a single graph and the half-life is obtained by averaging the rates for individual species. This practice reduces the time that single solutions must age, thereby reducing problems caused by evaporation and decreasing the chance of contamination in the test solutions. It also detects changes in rates of detoxification for different concentrations of toxicants.

A difficulty sometimes arises in determining the proper time to bioassay the aged solutions, especially without prior knowledge of the toxicant's persistence. After the initial test in method A, however, the data can be plotted using the single percent concentration remaining and assuming 100 percent biological activity at the beginning of the test. The slope of the curve may be influenced in subsequent tests, but through a quick half-life approximation the activity can be predicted for longer aging periods and more sensitive species. Following the tests of more sensitive species, the curve is redrawn for best fit to all of the calculated values.

Method A is limited by the number of differentially sensitive organisms available. In fact, to more accurately derive the half-life by this method, three or more values are desirable to establish the slope of the curve. Several of these values may be obtained using one species, although more than one species should be used in deriving the half-life curve. The model presents a reduction in toxicity by a factor of 1,000 using 10 different aging periods, but experimental chemicals frequently are not that differentially toxic nor are that many species available.

Advantages of method B are that only one species is required, fewer reference tests are necessary, and the total time and facility requirements are less. Both methods are estimates because the values derived are taken from a curve representing statistical calculations on the tolerance of biological organisms. The plotted values are experimental and do not necessarily occur on the curve. In method A the variation is among different species, whereas in method B the variation is among different tests of the same species. For better results, several species could be tested in method B and values could be averaged.

Neither method is intended to account for absorption and adsorption of test chemicals to different kinds of bioassay vessels or for complexing to organic matter found in different waters. Also, they do not differentiate between chemical degradation and biological inactivation. The methods estimate the available total activity of a chemical and its degradation products under defined test media conditions.

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Fish Control Laboratories
Bureau of Sport Fisheries and Wildlife
U.S. Department of the Interior
P.O. Box 862
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